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* * * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 15:42:45 ON 21 JUN 2000

=> fil .bec

COST IN U.S. DOLLARS                                        SINCE FILE      TOTAL
                                                               ENTRY    SESSION
FULL ESTIMATED COST                                             0.60       0.60

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO' ENTERED AT 15:44:57 ON 21 JUN 2000
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

10 FILES IN THE FILE LIST

=> s carbon(2a)(flux or flow)

FILE 'MEDLINE'
        165595 CARBON
         17572 FLUX
        253492 FLOW
L1         511 CARBON(2A)(FLUX OR FLOW)

FILE 'SCISEARCH'
        223808 CARBON
         85096 FLUX
        387138 FLOW
L2        1852 CARBON(2A)(FLUX OR FLOW)

FILE 'LIFESCI'
         34804 CARBON
          8025 FLUX
         35825 FLOW
L3         735 CARBON(2A)(FLUX OR FLOW)

FILE 'BIOTECHDS'
          8112 CARBON
          1107 FLUX
          9793 FLOW
L4         165 CARBON(2A)(FLUX OR FLOW)

FILE 'BIOSIS'
        199958 CARBON
         35451 FLUX
        272543 FLOW
L5        2389 CARBON(2A)(FLUX OR FLOW)

FILE 'EMBASE'
        106181 CARBON
         20902 FLUX
        264832 FLOW
L6         639 CARBON(2A)(FLUX OR FLOW)

FILE 'HCAPLUS'
        733260 CARBON
        168322 FLUX
        521084 FLOW
L7        3658 CARBON(2A)(FLUX OR FLOW)
```

```
FILE 'NTIS'
         66895 CARBON
         34694 FLUX
        156247 FLOW
L8         234 CARBON(2A)(FLUX OR FLOW)

FILE 'ESBIOBASE'
         31445 CARBON
          8657 FLUX
         47993 FLOW
L9         562 CARBON(2A)(FLUX OR FLOW)

FILE 'BIOTECHNO'
         25115 CARBON
          5096 FLUX
         34964 FLOW
L10        350 CARBON(2A)(FLUX OR FLOW)

TOTAL FOR ALL FILES
L11      11095 CARBON(2A)(FLUX OR FLOW)


=> s l11(6a)(modif? or alter? or increas?)

FILE 'MEDLINE'
        247875 MODIF?
        458113 ALTER?
       1365059 INCREAS?
L12         59 L1 (6A)(MODIF? OR ALTER? OR INCREAS?)

FILE 'SCISEARCH'
        314906 MODIF?
        412512 ALTER?
       1214418 INCREAS?
L13        116 L2 (6A)(MODIF? OR ALTER? OR INCREAS?)

FILE 'LIFESCI'
         69113 MODIF?
        125281 ALTER?
        359025 INCREAS?
L14         65 L3 (6A)(MODIF? OR ALTER? OR INCREAS?)

FILE 'BIOTECHDS'
         19630 MODIF?
         14000 ALTER?
         43431 INCREAS?
L15         30 L4 (6A)(MODIF? OR ALTER? OR INCREAS?)

FILE 'BIOSIS'
        275774 MODIF?
        703175 ALTER?
       1560260 INCREAS?
L16        141 L5 (6A)(MODIF? OR ALTER? OR INCREAS?)

FILE 'EMBASE'
        239269 MODIF?
        449466 ALTER?
       1353635 INCREAS?
L17         68 L6 (6A)(MODIF? OR ALTER? OR INCREAS?)
```

```
FILE 'HCAPLUS'
       588225 MODIF?
       543346 ALTER?
      2542153 INCREAS?
L18        141 L7 (6A)(MODIF? OR ALTER? OR INCREAS?)

FILE 'NTIS'
        91273 MODIF?
        83295 ALTER?
       165754 INCREAS?
L19          9 L8 (6A)(MODIF? OR ALTER? OR INCREAS?)

FILE 'ESBIOBASE'
        70732 MODIF?
       113735 ALTER?
       341323 INCREAS?
L20         52 L9 (6A)(MODIF? OR ALTER? OR INCREAS?)

FILE 'BIOTECHNO'
        60585 MODIF?
       105671 ALTER?
       273906 INCREAS?
L21         38 L10(6A)(MODIF? OR ALTER? OR INCREAS?)

TOTAL FOR ALL FILES
L22        719 L11(6A)(MODIF? OR ALTER? OR INCREAS?)

=> s (phosphoenolpyruvate or (phospho enol or phosphoenol)(w)pyruvate or
pep)(4a)(suppl#### or availab?)

FILE 'MEDLINE'
          5527 PHOSPHOENOLPYRUVATE
          2515 PHOSPHO
           596 ENOL
            55 PHOSPHO ENOL
                 (PHOSPHO(W)ENOL)
           206 PHOSPHOENOL
         20967 PYRUVATE
          2442 PEP
        270502 SUPPL####
        203666 AVAILAB?
L23         27 (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
               OR PEP)(4A)(SUPPL#### OR AVAILAB?)

FILE 'SCISEARCH'
          4795 PHOSPHOENOLPYRUVATE
          1961 PHOSPHO
          5686 ENOL
            58 PHOSPHO ENOL
                 (PHOSPHO(W)ENOL)
           187 PHOSPHOENOL
         15215 PYRUVATE
          2080 PEP
         78325 SUPPL####
        223789 AVAILAB?
L24         35 (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
               OR PEP)(4A)(SUPPL#### OR AVAILAB?)

FILE 'LIFESCI'
```

```
          1795 PHOSPHOENOLPYRUVATE
           923 "PHOSPHO"
           206 "ENOL"
            17 PHOSPHO ENOL
                 ("PHOSPHO"(W)"ENOL")
           101 PHOSPHOENOL
          4911 PYRUVATE
           720 PEP
         17032 SUPPL####
         63252 AVAILAB?
L25         14 (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
               OR PEP)(4A)(SUPPL#### OR AVAILAB?)

FILE 'BIOTECHDS'
           304 PHOSPHOENOLPYRUVATE
           142 PHOSPHO
           122 ENOL
             2 PHOSPHO ENOL
                 (PHOSPHO(W)ENOL)
            33 PHOSPHOENOL
          1358 PYRUVATE
           141 PEP
          5490 SUPPL####
          5616 AVAILAB?
L26          6 (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
               OR PEP)(4A)(SUPPL#### OR AVAILAB?)

FILE 'BIOSIS'
          6738 PHOSPHOENOLPYRUVATE
         55173 PHOSPHO
          1826 ENOL
           148 PHOSPHO ENOL
                 (PHOSPHO(W)ENOL)
          3598 PHOSPHOENOL
         31615 PYRUVATE
          3255 PEP
         82830 SUPPL####
        206349 AVAILAB?
L27         34 (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
               OR PEP)(4A)(SUPPL#### OR AVAILAB?)

FILE 'EMBASE'
          3759 PHOSPHOENOLPYRUVATE
          1882 "PHOSPHO"
          1423 "ENOL"
            38 PHOSPHO ENOL
                 ("PHOSPHO"(W)"ENOL")
           158 PHOSPHOENOL
         17239 PYRUVATE
          2258 PEP
         62096 SUPPL####
        207328 AVAILAB?
L28         27 (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
               OR PEP)(4A)(SUPPL#### OR AVAILAB?)

FILE 'HCAPLUS'
          9225 PHOSPHOENOLPYRUVATE
          6137 PHOSPHO
         13928 ENOL
            39 PHOSPHO ENOL
```

```
                (PHOSPHO(W)ENOL)
          493 PHOSPHOENOL
        36233 PYRUVATE
         4793 PEP
       146880 SUPPL####
       269875 AVAILAB?
L29         55 (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
               OR PEP)(4A)(SUPPL#### OR AVAILAB?)

FILE 'NTIS'
           36 PHOSPHOENOLPYRUVATE
           46 PHOSPHO
           74 ENOL
            0 PHOSPHO ENOL
                (PHOSPHO(W)ENOL)
            5 PHOSPHOENOL
          297 PYRUVATE
         1158 PEP
        81632 SUPPL####
       230225 AVAILAB?
L30         16 (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
               OR PEP)(4A)(SUPPL#### OR AVAILAB?)

FILE 'ESBIOBASE'
         1186 PHOSPHOENOLPYRUVATE
          834 PHOSPHO
          263 ENOL
           20 PHOSPHO ENOL
                (PHOSPHO(W)ENOL)
           45 PHOSPHOENOL
         3597 PYRUVATE
          572 PEP
        14145 SUPPL####
        50728 AVAILAB?
L31          9 (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
               OR PEP)(4A)(SUPPL#### OR AVAILAB?)

FILE 'BIOTECHNO'
         1947 PHOSPHOENOLPYRUVATE
          980 PHOSPHO
          149 ENOL
           17 PHOSPHO ENOL
                (PHOSPHO(W)ENOL)
           58 PHOSPHOENOL
         5154 PYRUVATE
          599 PEP
         9608 SUPPL####
        38606 AVAILAB?
L32         14 (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
               OR PEP)(4A)(SUPPL#### OR AVAILAB?)

TOTAL FOR ALL FILES
L33        237 (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W) PYRUVAT
               E OR PEP)(4A)(SUPPL#### OR AVAILAB?)

=> s phosphotransferase# or phospho transferase#

FILE 'MEDLINE'
        17143 PHOSPHOTRANSFERASE#
         2515 PHOSPHO
```

```
         34029 TRANSFERASE#
            14 PHOSPHO TRANSFERASE#
                 (PHOSPHO(W)TRANSFERASE#)
L34      17151 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

FILE 'SCISEARCH'
          3786 PHOSPHOTRANSFERASE#
          1961 PHOSPHO
         27571 TRANSFERASE#
            12 PHOSPHO TRANSFERASE#
                 (PHOSPHO(W)TRANSFERASE#)
L35       3796 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

FILE 'LIFESCI'
          2364 PHOSPHOTRANSFERASE#
           923 "PHOSPHO"
          9714 TRANSFERASE#
             7 PHOSPHO TRANSFERASE#
                 ("PHOSPHO"(W)TRANSFERASE#)
L36       2368 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

FILE 'BIOTECHDS'
          1618 PHOSPHOTRANSFERASE#
           142 PHOSPHO
          1549 TRANSFERASE#
             0 PHOSPHO TRANSFERASE#
                 (PHOSPHO(W)TRANSFERASE#)
L37       1618 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

FILE 'BIOSIS'
          5296 PHOSPHOTRANSFERASE#
         55173 PHOSPHO
         58857 TRANSFERASE#
          1749 PHOSPHO TRANSFERASE#
                 (PHOSPHO(W)TRANSFERASE#)
L38       6397 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

FILE 'EMBASE'
          7100 PHOSPHOTRANSFERASE#
          1882 "PHOSPHO"
         25872 TRANSFERASE#
             6 PHOSPHO TRANSFERASE#
                 ("PHOSPHO"(W)TRANSFERASE#)
L39       7105 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

FILE 'HCAPLUS'
          6267 PHOSPHOTRANSFERASE#
          6137 PHOSPHO
         33257 TRANSFERASE#
             8 PHOSPHO TRANSFERASE#
                 (PHOSPHO(W)TRANSFERASE#)
L40       6274 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

FILE 'NTIS'
           126 PHOSPHOTRANSFERASE#
            46 PHOSPHO
           713 TRANSFERASE#
             0 PHOSPHO TRANSFERASE#
                 (PHOSPHO(W)TRANSFERASE#)
L41         126 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#
```

```
FILE 'ESBIOBASE'
          2511 PHOSPHOTRANSFERASE#
           834 PHOSPHO
         19078 TRANSFERASE#
             5 PHOSPHO TRANSFERASE#
                 (PHOSPHO(W)TRANSFERASE#)
L42       2514 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

FILE 'BIOTECHNO'
          4633 PHOSPHOTRANSFERASE#
           980 PHOSPHO
         11638 TRANSFERASE#
             4 PHOSPHO TRANSFERASE#
                 (PHOSPHO(W)TRANSFERASE#)
L43       4635 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

TOTAL FOR ALL FILES
L44      51984 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

=> s l44 and l11

FILE 'MEDLINE'
L45         13 L34 AND L1

FILE 'SCISEARCH'
L46         20 L35 AND L2

FILE 'LIFESCI'
L47          7 L36 AND L3

FILE 'BIOTECHDS'
L48          5 L37 AND L4

FILE 'BIOSIS'
L49         11 L38 AND L5

FILE 'EMBASE'
L50          8 L39 AND L6

FILE 'HCAPLUS'
L51         12 L40 AND L7

FILE 'NTIS'
L52          1 L41 AND L8

FILE 'ESBIOBASE'
L53          8 L42 AND L9

FILE 'BIOTECHNO'
L54          8 L43 AND L10

TOTAL FOR ALL FILES
L55         93 L44 AND L11

=> s l44(8a)(delet? or inactivat?)

FILE 'MEDLINE'
         87427 DELET?
         76530 INACTIVAT?
```

```
L56         104 L34(8A)(DELET? OR INACTIVAT?)

FILE 'SCISEARCH'
         71717 DELET?
         58968 INACTIVAT?
L57          46 L35(8A)(DELET? OR INACTIVAT?)

FILE 'LIFESCI'
         40163 DELET?
         29342 INACTIVAT?
L58          61 L36(8A)(DELET? OR INACTIVAT?)

FILE 'BIOTECHDS'
          7050 DELET?
          5581 INACTIVAT?
L59          39 L37(8A)(DELET? OR INACTIVAT?)

FILE 'BIOSIS'
         83620 DELET?
         85386 INACTIVAT?
L60         109 L38(8A)(DELET? OR INACTIVAT?)

FILE 'EMBASE'
         73850 DELET?
         68050 INACTIVAT?
L61          73 L39(8A)(DELET? OR INACTIVAT?)

FILE 'HCAPLUS'
         79915 DELET?
         96594 INACTIVAT?
L62         139 L40(8A)(DELET? OR INACTIVAT?)

FILE 'NTIS'
          4028 DELET?
          1911 INACTIVAT?
L63           0 L41(8A)(DELET? OR INACTIVAT?)

FILE 'ESBIOBASE'
         32736 DELET?
         21336 INACTIVAT?
L64          15 L42(8A)(DELET? OR INACTIVAT?)

FILE 'BIOTECHNO'
         52605 DELET?
         29669 INACTIVAT?
L65          46 L43(8A)(DELET? OR INACTIVAT?)

TOTAL FOR ALL FILES
L66         632 L44(8A)(DELET? OR INACTIVAT?)

=> s l66 and transport?

FILE 'MEDLINE'
        199769 TRANSPORT?
L67           9 L56 AND TRANSPORT?

FILE 'SCISEARCH'
        284924 TRANSPORT?
L68           1 L57 AND TRANSPORT?
```

```
FILE 'LIFESCI'
        54644 TRANSPORT?
L69         2 L58 AND TRANSPORT?

FILE 'BIOTECHDS'
         3396 TRANSPORT?
L70         1 L59 AND TRANSPORT?

FILE 'BIOSIS'
       880765 TRANSPORT?
L71        11 L60 AND TRANSPORT?

FILE 'EMBASE'
       202138 TRANSPORT?
L72         9 L61 AND TRANSPORT?

FILE 'HCAPLUS'
       494433 TRANSPORT?
L73        16 L62 AND TRANSPORT?

FILE 'NTIS'
       126980 TRANSPORT?
L74         0 L63 AND TRANSPORT?

FILE 'ESBIOBASE'
       110606 TRANSPORT?
L75         1 L64 AND TRANSPORT?

FILE 'BIOTECHNO'
         57183 TRANSPORT?
L76         3 L65 AND TRANSPORT?

TOTAL FOR ALL FILES
L77        53 L66 AND TRANSPORT?

=> s l44 and glucose

FILE 'MEDLINE'
       210335 GLUCOSE
L78      2132 L34 AND GLUCOSE

FILE 'SCISEARCH'
       131091 GLUCOSE
L79       676 L35 AND GLUCOSE

FILE 'LIFESCI'
         33398 GLUCOSE
L80        418 L36 AND GLUCOSE

FILE 'BIOTECHDS'
         24919 GLUCOSE
L81         81 L37 AND GLUCOSE

FILE 'BIOSIS'
       217611 GLUCOSE
L82      1063 L38 AND GLUCOSE

FILE 'EMBASE'
       169880 GLUCOSE
L83        851 L39 AND GLUCOSE
```

```
FILE 'HCAPLUS'
        245956 GLUCOSE
L84       1184 L40 AND GLUCOSE

FILE 'NTIS'
          2781 GLUCOSE
L85          9 L41 AND GLUCOSE

FILE 'ESBIOBASE'
         32091 GLUCOSE
L86        334 L42 AND GLUCOSE

FILE 'BIOTECHNO'
         32114 GLUCOSE
L87        518 L43 AND GLUCOSE

TOTAL FOR ALL FILES
L88       7266 L44 AND GLUCOSE

=> s l66 and l88

FILE 'MEDLINE'
L89          15 L56 AND L78

FILE 'SCISEARCH'
L90           4 L57 AND L79

FILE 'LIFESCI'
L91           6 L58 AND L80

FILE 'BIOTECHDS'
L92           4 L59 AND L81

FILE 'BIOSIS'
L93          16 L60 AND L82

FILE 'EMBASE'
L94          14 L61 AND L83

FILE 'HCAPLUS'
L95          19 L62 AND L84

FILE 'NTIS'
L96           0 L63 AND L85

FILE 'ESBIOBASE'
L97           3 L64 AND L86

FILE 'BIOTECHNO'
L98           7 L65 AND L87

TOTAL FOR ALL FILES
L99          88 L66 AND L88

=> s l88 and transport

FILE 'MEDLINE'
        169094 TRANSPORT
L100       590 L78 AND TRANSPORT
```

```
FILE 'SCISEARCH'
       252462 TRANSPORT
L101      303 L79 AND TRANSPORT

FILE 'LIFESCI'
        44285 TRANSPORT
L102      165 L80 AND TRANSPORT

FILE 'BIOTECHDS'
         2625 TRANSPORT
L103       16 L81 AND TRANSPORT

FILE 'BIOSIS'
       853558 TRANSPORT
L104      356 L82 AND TRANSPORT

FILE 'EMBASE'
       183212 TRANSPORT
L105      373 L83 AND TRANSPORT

FILE 'HCAPLUS'
       445874 TRANSPORT
L106      486 L84 AND TRANSPORT

FILE 'NTIS'
        73937 TRANSPORT
L107        3 L85 AND TRANSPORT

FILE 'ESBIOBASE'
       103397 TRANSPORT
L108      162 L86 AND TRANSPORT

FILE 'BIOTECHNO'
        50543 TRANSPORT
L109      248 L87 AND TRANSPORT

TOTAL FOR ALL FILES
L110     2702 L88 AND TRANSPORT

=> s l110 and (phosphoenolpyruvate or (phospho enol or phosphoenol)(w)pyruvate or
pep)

FILE 'MEDLINE'
          5527 PHOSPHOENOLPYRUVATE
          2515 PHOSPHO
           596 ENOL
            55 PHOSPHO ENOL
                 (PHOSPHO(W)ENOL)
           206 PHOSPHOENOL
         20967 PYRUVATE
           230 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
          2442 PEP
L111       310 L100 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W
               )PYRUVATE OR PEP)

FILE 'SCISEARCH'
          4795 PHOSPHOENOLPYRUVATE
          1961 PHOSPHO
          5686 ENOL
```

```
            58 PHOSPHO ENOL
                 (PHOSPHO(W)ENOL)
           187 PHOSPHOENOL
         15215 PYRUVATE
           228 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
          2080 PEP
L112       206 L101 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W
               )PYRUVATE OR PEP)

FILE 'LIFESCI'
          1795 PHOSPHOENOLPYRUVATE
           923 "PHOSPHO"
           206 "ENOL"
            17 PHOSPHO ENOL
                 ("PHOSPHO"(W)"ENOL")
           101 PHOSPHOENOL
          4911 PYRUVATE
           108 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
           720 PEP
L113       120 L102 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W
               )PYRUVATE OR PEP)

FILE 'BIOTECHDS'
           304 PHOSPHOENOLPYRUVATE
           142 PHOSPHO
           122 ENOL
             2 PHOSPHO ENOL
                 (PHOSPHO(W)ENOL)
            33 PHOSPHOENOL
          1358 PYRUVATE
            32 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
           141 PEP
L114        10 L103 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W
               )PYRUVATE OR PEP)

FILE 'BIOSIS'
          6738 PHOSPHOENOLPYRUVATE
         55173 PHOSPHO
          1826 ENOL
           148 PHOSPHO ENOL
                 (PHOSPHO(W)ENOL)
          3598 PHOSPHOENOL
         31615 PYRUVATE
          3685 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
          3255 PEP
L115       241 L104 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W
               )PYRUVATE OR PEP)

FILE 'EMBASE'
          3759 PHOSPHOENOLPYRUVATE
          1882 "PHOSPHO"
          1423 "ENOL"
            38 PHOSPHO ENOL
                 ("PHOSPHO"(W)"ENOL")
           158 PHOSPHOENOL
         17239 PYRUVATE
           179 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
          2258 PEP
L116       234 L105 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W
               )PYRUVATE OR PEP)
```

```
FILE 'HCAPLUS'
          9225 PHOSPHOENOLPYRUVATE
          6137 PHOSPHO
         13928 ENOL
            39 PHOSPHO ENOL
               (PHOSPHO(W)ENOL)
           493 PHOSPHOENOL
         36233 PYRUVATE
           466 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
          4793 PEP
L117       362 L106 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W
               )PYRUVATE OR PEP)

FILE 'NTIS'
            36 PHOSPHOENOLPYRUVATE
            46 PHOSPHO
            74 ENOL
             0 PHOSPHO ENOL
               (PHOSPHO(W)ENOL)
             5 PHOSPHOENOL
           297 PYRUVATE
             3 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
          1158 PEP
L118          3 L107 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W
               )PYRUVATE OR PEP)

FILE 'ESBIOBASE'
          1186 PHOSPHOENOLPYRUVATE
           834 PHOSPHO
           263 ENOL
            20 PHOSPHO ENOL
               (PHOSPHO(W)ENOL)
            45 PHOSPHOENOL
          3597 PYRUVATE
            63 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
           572 PEP
L119        84 L108 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W
               )PYRUVATE OR PEP)

FILE 'BIOTECHNO'
          1947 PHOSPHOENOLPYRUVATE
           980 PHOSPHO
           149 ENOL
            17 PHOSPHO ENOL
               (PHOSPHO(W)ENOL)
            58 PHOSPHOENOL
          5154 PYRUVATE
            69 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
           599 PEP
L120       157 L109 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W
               )PYRUVATE OR PEP)

TOTAL FOR ALL FILES
L121      1727 L110 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W
               ) PYRUVATE OR PEP)

=> s l121 and mut/q

FILE 'MEDLINE'
```

```
L122        194 L111 AND MUT/Q

FILE 'SCISEARCH'
L123        122 L112 AND MUT/Q

FILE 'LIFESCI'
L124         61 L113 AND MUT/Q

FILE 'BIOTECHDS'
L125          8 L114 AND MUT/Q

FILE 'BIOSIS'
L126        134 L115 AND MUT/Q

FILE 'EMBASE'
L127        131 L116 AND MUT/Q

FILE 'HCAPLUS'
L128        187 L117 AND MUT/Q

FILE 'NTIS'
L129          2 L118 AND MUT/Q

FILE 'ESBIOBASE'
L130         53 L119 AND MUT/Q

FILE 'BIOTECHNO'
L131         95 L120 AND MUT/Q

TOTAL FOR ALL FILES
L132        987 L121 AND MUT/Q

=> s l132 and (aromatic or shikimate)

FILE 'MEDLINE'
         20282 AROMATIC
           282 SHIKIMATE
L133         2 L122 AND (AROMATIC OR SHIKIMATE)

FILE 'SCISEARCH'
         67941 AROMATIC
           640 SHIKIMATE
L134         3 L123 AND (AROMATIC OR SHIKIMATE)

FILE 'LIFESCI'
         10616 AROMATIC
           228 SHIKIMATE
L135         2 L124 AND (AROMATIC OR SHIKIMATE)

FILE 'BIOTECHDS'
          4057 AROMATIC
            86 SHIKIMATE
L136         1 L125 AND (AROMATIC OR SHIKIMATE)

FILE 'BIOSIS'
         37167 AROMATIC
           957 SHIKIMATE
L137         2 L126 AND (AROMATIC OR SHIKIMATE)

FILE 'EMBASE'
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         32677 AROMATIC
           239 SHIKIMATE
L138         2 L127 AND (AROMATIC OR SHIKIMATE)

FILE 'HCAPLUS'
        127299 AROMATIC
        222721 AROM
        272472 AROMATIC
                 (AROMATIC OR AROM)
          1406 SHIKIMATE
L139         4 L128 AND (AROMATIC OR SHIKIMATE)

FILE 'NTIS'
         10987 AROMATIC
             8 SHIKIMATE
L140         0 L129 AND (AROMATIC OR SHIKIMATE)

FILE 'ESBIOBASE'
          8986 AROMATIC
           168 SHIKIMATE
L141         1 L130 AND (AROMATIC OR SHIKIMATE)

FILE 'BIOTECHNO'
          9121 AROMATIC
           158 SHIKIMATE
L142         2 L131 AND (AROMATIC OR SHIKIMATE)

TOTAL FOR ALL FILES
L143        19 L132 AND (AROMATIC OR SHIKIMATE)

=> s l22 and (phosphoenolpyruvate or (phospho enol or phosphoenol)(w)pyruvate or
pep)

FILE 'MEDLINE'
          5527 PHOSPHOENOLPYRUVATE
          2515 PHOSPHO
           596 ENOL
            55 PHOSPHO ENOL
                 (PHOSPHO(W)ENOL)
           206 PHOSPHOENOL
         20967 PYRUVATE
           230 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
          2442 PEP
L144         6 L12 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)
               PYRUVATE OR PEP)

FILE 'SCISEARCH'
          4795 PHOSPHOENOLPYRUVATE
          1961 PHOSPHO
          5686 ENOL
            58 PHOSPHO ENOL
                 (PHOSPHO(W)ENOL)
           187 PHOSPHOENOL
         15215 PYRUVATE
           228 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
          2080 PEP
L145         9 L13 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)
               PYRUVATE OR PEP)

FILE 'LIFESCI'
```

```
          1795 PHOSPHOENOLPYRUVATE
           923 "PHOSPHO"
           206 "ENOL"
            17 PHOSPHO ENOL
                 ("PHOSPHO"(W)"ENOL")
           101 PHOSPHOENOL
          4911 PYRUVATE
           108 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
           720 PEP
L146         8 L14 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)
               PYRUVATE OR PEP)

FILE 'BIOTECHDS'
           304 PHOSPHOENOLPYRUVATE
           142 PHOSPHO
           122 ENOL
             2 PHOSPHO ENOL
                 (PHOSPHO(W)ENOL)
            33 PHOSPHOENOL
          1358 PYRUVATE
            32 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
           141 PEP
L147         5 L15 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)
               PYRUVATE OR PEP)

FILE 'BIOSIS'
          6738 PHOSPHOENOLPYRUVATE
         55173 PHOSPHO
          1826 ENOL
           148 PHOSPHO ENOL
                 (PHOSPHO(W)ENOL)
          3598 PHOSPHOENOL
         31615 PYRUVATE
          3685 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
          3255 PEP
L148        14 L16 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)
               PYRUVATE OR PEP)

FILE 'EMBASE'
          3759 PHOSPHOENOLPYRUVATE
          1882 "PHOSPHO"
          1423 "ENOL"
            38 PHOSPHO ENOL
                 ("PHOSPHO"(W)"ENOL")
           158 PHOSPHOENOL
         17239 PYRUVATE
           179 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
          2258 PEP
L149         6 L17 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)
               PYRUVATE OR PEP)

FILE 'HCAPLUS'
          9225 PHOSPHOENOLPYRUVATE
          6137 PHOSPHO
         13928 ENOL
            39 PHOSPHO ENOL
                 (PHOSPHO(W)ENOL)
           493 PHOSPHOENOL
         36233 PYRUVATE
           466 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
```

```
         4793 PEP
L150       16 L18 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)
              PYRUVATE OR PEP)

FILE 'NTIS'
            36 PHOSPHOENOLPYRUVATE
            46 PHOSPHO
            74 ENOL
             0 PHOSPHO ENOL
               (PHOSPHO(W)ENOL)
             5 PHOSPHOENOL
           297 PYRUVATE
             3 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
          1158 PEP
L151        0 L19 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)
              PYRUVATE OR PEP)

FILE 'ESBIOBASE'
          1186 PHOSPHOENOLPYRUVATE
           834 PHOSPHO
           263 ENOL
            20 PHOSPHO ENOL
               (PHOSPHO(W)ENOL)
            45 PHOSPHOENOL
          3597 PYRUVATE
            63 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
           572 PEP
L152         6 L20 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)
              PYRUVATE OR PEP)

FILE 'BIOTECHNO'
          1947 PHOSPHOENOLPYRUVATE
           980 PHOSPHO
           149 ENOL
            17 PHOSPHO ENOL
               (PHOSPHO(W)ENOL)
            58 PHOSPHOENOL
          5154 PYRUVATE
            69 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
           599 PEP
L153         6 L21 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)
              PYRUVATE OR PEP)

TOTAL FOR ALL FILES
L154        76 L22 AND (PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)
               PYRUVATE OR PEP)

=> s l22 and glucose

FILE 'MEDLINE'
        210335 GLUCOSE
L155        22 L12 AND GLUCOSE

FILE 'SCISEARCH'
        131091 GLUCOSE
L156        23 L13 AND GLUCOSE

FILE 'LIFESCI'
         33398 GLUCOSE
L157        13 L14 AND GLUCOSE
```

```
FILE 'BIOTECHDS'
         24919 GLUCOSE
L158        13 L15 AND GLUCOSE

FILE 'BIOSIS'
        217611 GLUCOSE
L159        31 L16 AND GLUCOSE

FILE 'EMBASE'
        169880 GLUCOSE
L160        28 L17 AND GLUCOSE

FILE 'HCAPLUS'
        245956 GLUCOSE
L161        28 L18 AND GLUCOSE

FILE 'NTIS'
          2781 GLUCOSE
L162         0 L19 AND GLUCOSE

FILE 'ESBIOBASE'
         32091 GLUCOSE
L163        14 L20 AND GLUCOSE

FILE 'BIOTECHNO'
         32114 GLUCOSE
L164        18 L21 AND GLUCOSE

TOTAL FOR ALL FILES
L165       190 L22 AND GLUCOSE

=> s (l33 or l55 or l77 or l99 or l132 or l143 or l154 or l165) and py=<1995
range=1998,

FILE 'MEDLINE'
          7783 PY=<1995
L166         0 (L23 OR L45 OR L67 OR L89 OR L122 OR L133 OR L144 OR L155) AND
               PY=<1995

FILE 'SCISEARCH'
            76 PY=<1995
L167         0 (L24 OR L46 OR L68 OR L90 OR L123 OR L134 OR L145 OR L156) AND
               PY=<1995

FILE 'LIFESCI'
          2457 PY=<1995
L168         0 (L25 OR L47 OR L69 OR L91 OR L124 OR L135 OR L146 OR L157) AND
               PY=<1995

FILE 'BIOTECHDS'
            25 PY=<1995
                 (PY=<1995)
L169         0 (L26 OR L48 OR L70 OR L92 OR L125 OR L136 OR L147 OR L158) AND
               PY=<1995

FILE 'BIOSIS'
          1354 PY=<1995
L170         0 (L27 OR L49 OR L71 OR L93 OR L126 OR L137 OR L148 OR L159) AND
               PY=<1995
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```
FILE 'EMBASE'
          81 PY=<1995
L171       0 (L28 OR L50 OR L72 OR L94 OR L127 OR L138 OR L149 OR L160) AND
             PY=<1995

FILE 'HCAPLUS'
        6507 PY=<1995
L172       0 (L29 OR L51 OR L73 OR L95 OR L128 OR L139 OR L150 OR L161) AND
             PY=<1995

FILE 'NTIS'
       47041 PY=<1995
L173       0 (L30 OR L52 OR L74 OR L96 OR L129 OR L140 OR L151 OR L162) AND
             PY=<1995

FILE 'ESBIOBASE'
           0 PY=<1995
L174       0 (L31 OR L53 OR L75 OR L97 OR L130 OR L141 OR L152 OR L163) AND
             PY=<1995

FILE 'BIOTECHNO'
      845083 PY=<1995
L175      75 (L32 OR L54 OR L76 OR L98 OR L131 OR L142 OR L153 OR L164) AND
             PY=<1995

TOTAL FOR ALL FILES
L176      75 (L33 OR L55 OR L77 OR L99 OR L132 OR L143 OR L154 OR L165) AND
             PY=<1995

=> dup rem l176

PROCESSING COMPLETED FOR L176
L177       75 DUP REM L176 (0 DUPLICATES REMOVED)

=> d tot


L177  ANSWER 1 OF 75  BIOTECHNO  COPYRIGHT 2000 Elsevier Science B.V.
TI    Glucose kinase-dependent catabolite repression in
      Staphylococcus xylosus
SO    Journal of Bacteriology, (1995), 177/21 (6144-6152)
      CODEN: JOBAAY  ISSN: 0021-9193
AU    Wagner E.; Marcandier S.; Egeter O.; Deutscher J.; Gotz F.; Bruckner R.
AN    1995:25326230   BIOTECHNO

L177  ANSWER 2 OF 75  BIOTECHNO  COPYRIGHT 2000 Elsevier Science B.V.
TI    Regulation of sugar transport via the multiple sugar metabolism
      operon of Streptococcus mutans by the
    phosphoenolpyruvate phosphotransferase system
SO    Journal of Bacteriology, (1995), 177/19 (5704-5706)
      CODEN: JOBAAY  ISSN: 0021-9193
AU    Cvitkovitch D.G.; Boyd D.A.; Hamilton I.R.
AN    1995:26028884   BIOTECHNO

L177  ANSWER 3 OF 75  BIOTECHNO  COPYRIGHT 2000 Elsevier Science B.V.
TI    Use of feedback-resistant threonine dehydratases of Corynebacterium
      glutamicum to increase carbon flux towards
      L-isoleucine
SO    Applied and Environmental Microbiology, (1995), 61/12
```

(4315-4320)
CODEN: AEMIDF ISSN: 0099-2240
AU Morbach S.; Sahm H.; Eggeling L.
AN 1995:25361670 BIOTECHNO

L177 ANSWER 4 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Regulation of ATP-dependent P-(Ser)-HPr formation in Streptococcus **mutans** and Streptococcus salivarius
SO Journal of Bacteriology, (**1995**), 177/10 (2751-2759)
CODEN: JOBAAY ISSN: 0021-9193
AU Thevenot T.; Brochu D.; Vadeboncoeur C.; Hamilton I.R.
AN 1995:25148797 BIOTECHNO

L177 ANSWER 5 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Sequence, expression, and function of the gene for the nonphosphorylating, NADP-dependent glyceraldehyde-3-phosphate dehydrogenase of Streptococcus **mutans**
SO Journal of Bacteriology, (**1995**), 177/10 (2622-2627)
CODEN: JOBAAY ISSN: 0021-9193
AU Boyd D.A.; Cvitkovitch D.G.; Hamilton I.R.
AN 1995:25148781 BIOTECHNO

L177 ANSWER 6 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Regulation of the lactose **phosphotransferase** system of Streptococcus bovis by **glucose**: Independence of inducer exclusion and expulsion mechanisms
SO Microbiology, (**1995**), 141/9 (2261-2269)
CODEN: MROBEO ISSN: 1350-0872
AU Cook G.M.; Kearns D.B.; Russell J.B.; Reizer J.; Saier Jr. M.H.
AN 1995:25303431 BIOTECHNO

L177 ANSWER 7 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI **Glucose transport** by a **mutant** of Streptococcus **mutans** unable to accumulate sugars via the **phosphoenolpyruvate phosphotransferase** system
SO Journal of Bacteriology, (**1995**), 177/9 (2251-2258)
CODEN: JOBAAY ISSN: 0021-9193
AU Cvitkovitch D.G.; Boyd D.A.; Thevenot T.; Hamilton I.R.
AN 1995:25144127 BIOTECHNO

L177 ANSWER 8 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Regulation of bacterial sugar-H.sup.+ symport by **phosphoenolpyruvate**-dependent enzyme I/HPr-mediated phosphorylation
SO Proceedings of the National Academy of Sciences of the United States of America, (**1995**), 92/3 (778-782)
CODEN: PNASA6 ISSN: 0027-8424
AU Poolman B.; Knol J.; Mollet B.; Nieuwenhuis B.; Sulter G.
AN 1995:25048792 BIOTECHNO

L177 ANSWER 9 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI In Saccharomyces cerevisiae deletion of phosphoglucose isomerase can be suppressed by increased activities of enzymes of the hexose monophosphate pathway
SO Microbiology, (**1995**), 141/2 (385-391)
CODEN: MROBEO ISSN: 1350-0872
AU Dickinson J.R.; Sobanski M.A.; Hewlins M.J.E.
AN 1995:25070660 BIOTECHNO

L177 ANSWER 10 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.

TI How neutral red **modified carbon** and electron **flow** in Clostridium acetobutylicum grown in chemostat culture at neutral pH
SO FEMS Microbiology Reviews, (**1995**), 16/2-3 (151-162)
CODEN: FMREE4 ISSN: 0168-6445
AU Girbal L.; Vasconcelos I.; Saint-Amans S.; Soucaille P.
AN 1995:25068038 BIOTECHNO

L177 ANSWER 11 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Improved strains of recombinant Escherichia coli for ethanol production from sugar mixtures
SO Applied Microbiology and Biotechnology, (**1995**), 43/1 (70-75)
CODEN: AMBIDG ISSN: 0175-7598
AU Lindsay S.E.; Bothast R.J.; Ingram L.O.
AN 1995:25138872 BIOTECHNO

L177 ANSWER 12 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Inhibition of the **phosphoenolpyruvate**:lactose **phosphotransferase** system and activation of a cytoplasmic sugar-phosphate phosphatase in Lactococcus lactis by ATP-dependent metabolite-activated phosphorylation of serine 46 in the phosphocarrier protein HPr
SO Journal of Biological Chemistry, (**1994**), 269/16 (11837-11844)
CODEN: JBCHA3 ISSN: 0021-9258
AU Jing Jing Ye; Reizer J.; Cui X.; Saier Jr. M.H.
AN 1994:24196670 BIOTECHNO

L177 ANSWER 13 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI The role of phosphoenolpyruvate in the simultaneous uptake of fructose and 2-deoxyglucose by Escherichia coli
SO Proceedings of the National Academy of Sciences of the United States of America, (**1994**), 91/23 (11080-11083)
CODEN: PNASA6 ISSN: 0027-8424
AU Kornberg H.; Lambourne L.T.M.
AN 1994:24349629 BIOTECHNO

L177 ANSWER 14 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Characterization of a **glucose transport** system in Vibrio parahaemolyticus
SO Journal of Bacteriology, (**1994**), 176/23 (7378-7382)
CODEN: JOBAAY ISSN: 0021-9193
AU Sarker R.I.; Ogawa W.; Tsuda M.; Tanaka S.; Tsuchiya T.
AN 1994:24364067 BIOTECHNO

L177 ANSWER 15 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Engineering of Escherichia coli central metabolism for **aromatic** metabolite production with near theoretical yield
SO Applied and Environmental Microbiology, (**1994**), 60/11 (3903-3908)
CODEN: AEMIDF ISSN: 0099-2240
AU Patnaik R.; Liao J.C.
AN 1994:24335837 BIOTECHNO

L177 ANSWER 16 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Regulation of 2-deoxyglucose phosphate accumulation in Lactococcus lactis vesicles by metabolite-activated, ATP-dependent phosphorylation of serine-46 in HPr of the **phosphotransferase** system
SO Microbiology, (**1994**), 140/12 (3421-3429)
CODEN: MROBEO ISSN: 1350-0872
AU Ye J.J.; Reizer J.; Saier Jr. M.H.

AN 1994:25020629 BIOTECHNO

L177 ANSWER 17 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Loss of protein kinase-catalyzed phosphorylation of HPr, a phosphocarrier protein of the **phosphotransferase** system, by **mutation** of the ptsH gene confers catabolite repression resistance to several catabolic genes of Bacillus subtilis
SO Journal of Bacteriology, (**1994**), 176/11 (3336-3344)
CODEN: JOBAAY ISSN: 0021-9193
AU Deutscher J.; Reizer J.; Fischer C.; Galinier A.; Saier Jr. M.H.; Steinmetz M.
AN 1994:24166726 BIOTECHNO

L177 ANSWER 18 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Vesicles prepared from Streptococcus **mutans** demonstrate the presence of a second **glucose transport** system
SO Microbiology, (**1994**), 140/10 (2639-2648)
CODEN: MROBEO ISSN: 1350-0872
AU Buckley N.D.; Hamilton I.R.
AN 1994:24326662 BIOTECHNO

L177 ANSWER 19 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Mechanism of catabolite repression of tryptophanase synthesis in Escherichia coli
SO Microbiology, (**1994**), 140/8 (2125-2134)
CODEN: MROBEO ISSN: 1350-0872
AU Isaacs Jr H.; Chao D.; Yanofsky C.; Saier Jr M.H.
AN 1994:24259050 BIOTECHNO

L177 ANSWER 20 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Genetic regulation of fructosyltransferase in Streptococcus mutans
SO Infection and Immunity, (**1994**), 62/4 (1241-1251)
CODEN: INFIBR ISSN: 0019-9567
AU Kiska D.L.; Macrina F.L.
AN 1994:24110816 BIOTECHNO

L177 ANSWER 21 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Sequence and expression of the genes for HPr (ptsH) and enzyme I (ptsI) of the **phosphoenolpyruvate**-dependent **phosphotransferase transport** system from Streptococcus **mutans**
SO Infection and Immunity, (**1994**), 62/4 (1156-1165)
CODEN: INFIBR ISSN: 0019-9567
AU Boyd D.A.; Cvitkovitch D.G.; Hamilton I.R.
AN 1994:24110804 BIOTECHNO

L177 ANSWER 22 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI **Glucose transport** by the **phosphoenolpyruvate** : Mannose **phosphotransferase** system in Lactobacillus casei ATCC 393 and its role in carbon catabolite repression
SO Microbiology, (**1994**), 140/5 (1141-1149)
CODEN: MROBEO ISSN: 1350-0872
AU Veyrat A.; Monedero V.; Perez-Martinez G.
AN 1994:24157864 BIOTECHNO

L177 ANSWER 23 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI The **glucose**-starvation stimulon of Escherichia coli: Induced and repressed synthesis of enzymes of central metabolic pathways and role of acetyl phosphate in gene expression and starvation survival
SO Molecular Microbiology, (**1994**), 12/5 (833-843)
CODEN: MOMIEE ISSN: 0950-382X

AU Nystrom T.
AN 1994:24214476 BIOTECHNO

L177 ANSWER 24 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Alteration of the biochemical valves in the central metabolism of escherichia coli
SO Annals of the New York Academy of Sciences, (**1994**), 745/- (21-34)
CODEN: ANYAA0 ISSN: 0077-8923
AU Liao J.C.; Chao Y.-P.; Patnaik R.
AN 1994:26005842 BIOTECHNO

L177 ANSWER 25 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Alteration of growth yield by overexpression of **phosphoenolpyruvate** carboxylase and **phosphoenolpyruvate** carboxykinase in Escherichia coli
SO Applied and Environmental Microbiology, (**1993**), 59/12 (4261-4265)
CODEN: AEMIDF ISSN: 0099-2240
AU Chao Y.-P.; Liao J.C.
AN 1993:23361415 BIOTECHNO

L177 ANSWER 26 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Effects of N-acetylglucosamine on carbohydrate fermentation by Streptococcus **mutans** NCTC 10449 and Streptococcus sobrinus SL-1
SO Infection and Immunity, (**1993**), 61/1 (295-302)
CODEN: INFIBR ISSN: 0019-9567
AU Homer K.A.; Patel R.; Beighton D.
AN 1993:23015430 BIOTECHNO

L177 ANSWER 27 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Functional interactions between proteins of the **phosphoenolpyruvate**:sugar **phosphotransferase** systems of Bacillus subtilis and Escherichia coli
SO Journal of Biological Chemistry, (**1992**), 267/13 (9158-9169)
CODEN: JBCHA3 ISSN: 0021-9258
AU Reizer J.; Sutrina S.L.; Wu L.-F.; Deutscher J.; Reddy P.; Saier Jr. M.H.
AN 1992:22282071 BIOTECHNO

L177 ANSWER 28 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Lithium inhibits hepatic gluconeogenesis and **phosphoenolpyruvate** carboxykinase gene expression
SO Journal of Biological Chemistry, (**1992**), 267/5 (2888-2893)
CODEN: JBCHA3 ISSN: 0021-9258
AU Bosch F.; Rodriguez-Gil J.E.; Hatzoglou M.; Gomez-Foix A.M.; Hanson R.W.
AN 1992:22306506 BIOTECHNO

L177 ANSWER 29 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Analysis of **mutations** that uncouple **transport** from phosphorylation in enzyme II(Glc) of the Escherichia coli **phosphoenolpyruvate**-dependent **phosphotransferase** system
SO Journal of Bacteriology, (**1992**), 174/9 (2843-2850)
CODEN: JOBAAY ISSN: 0021-9193
AU Ruijter G.J.G.; Van Meurs G.; Verwey M.A.; Postma P.W.; Van Dam K.
AN 1992:22184632 BIOTECHNO

L177 ANSWER 30 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Factors affecting the manganese and iron activation of the phosphoenolpyruvate carboxykinase isozymes from rabbit
SO Biochimica et Biophysica Acta - General Subjects, (**1992**),

1156/1 (85-91)
CODEN: BBGSB3 ISSN: 0304-4165
AU lambeth D.O.; Muhonen W.W.; Jacoby G.H.; Ray P.D.
AN 1992:23004910 BIOTECHNO

L177 ANSWER 31 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Insertion of the mannitol permease into the membrane of Escherichia coli: Possible involvement of an N-terminal amphiphilic sequence
SO Journal of Biological Chemistry, (**1991**), 266/27 (17863-17871)
CODEN: JBCHA3 ISSN: 0021-9258
AU Yamada Y.; Chang Y.-Y.; Daniels G.A.; Wu L.-F.; Tomich J.M.; Yamada M.; Saier Jr. M.H.
AN 1991:21332664 BIOTECHNO

L177 ANSWER 32 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Secondary structure of the phosphocarrier protein III(Glc), a signal-transducing protein from Escherichia coli, determined by heteronuclear three- dimensional NMR spectroscopy
SO Proceedings of the National Academy of Sciences of the United States of America, (**1991**), 88/8 (3479-3483)
CODEN: PNASA6 ISSN: 0027-8424
AU Pelton J.G.; Torchia D.A.; Meadow N.D.; Wong C.-Y.; Roseman S.
AN 1991:22072878 BIOTECHNO

L177 ANSWER 33 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI The **glucose** permease of the **phosphotransferase** system of Bacillus subtilis: Evidence for II(Glc) and III(Glc) domains
SO Molecular Microbiology, (**1991**), 5/5 (1241-1249)
CODEN: MOMIEE ISSN: 0950-382X
AU Gonzy-Treboul G.; De Waard J.H.; Zagorec M.; Postma P.W.
AN 1991:21190827 BIOTECHNO

L177 ANSWER 34 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Release of **glucose**-mediated catabolite repression due to a defect in the membrane fraction of **phosphoenolpyruvate**: Mannose **phosphotransferase** system in Pediococcus halophilus
SO Archives of Microbiology, (**1991**), 155/6 (517-520)
CODEN: AMICCW ISSN: 0302-8933
AU Abe K.; Uchida K.
AN 1991:21206538 BIOTECHNO

L177 ANSWER 35 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI **Phosphotransferase**-dependent **glucose transport** in Corynebacterium glutamicum
SO Journal of Applied Bacteriology, (**1991**), 71/6 (517-523)
CODEN: JABAA4 ISSN: 0021-8847
AU Malin G.M.; Bourd G.I.
AN 1991:22010769 BIOTECHNO

L177 ANSWER 36 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Energetics of **glucose** uptake in a Salmonella typhimurium **mutant** containing uncoupled enzyme II(Glc)
SO Archives of Microbiology, (**1991**), 155/3 (234-237)
CODEN: AMICCW ISSN: 0302-8933
AU Ruijter G.J.G.; Postma P.W.; Van Dam K.
AN 1991:21108410 BIOTECHNO

L177 ANSWER 37 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI A unique zinc finger protein is associated preferentially with active ecdysone-responsive loci in Drosophila

SO Genes and Development, (**1991**), 5/2 (188-200)
CODEN: GEDEEP ISSN: 0890-9369
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CODEN: JOBAAY
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CODEN: INFIBR
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CODEN: CJMIAZ
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CODEN: EJBCAI
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CODEN: JOBAAY
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L177 ANSWER 73 OF 75 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
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CODEN: JOBAAY
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CODEN: JOBAAY
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	L #	Hits	Search Text	DBs	Time Stamp
1	L1	2792	carbon near2 (flux or flow)	USPAT	2000/06/21 09:22
2	L2	133	1 near4 (modif$8 or alter$8 or increas$8)	USPAT	2000/06/21 09:25
3	L3	100	(phosphoenol adj pyruvate or pep or phosphoenolpyruvate or phospho adj enol adj pyruvate) near4 (suppl$4 or availab$8)	USPAT	2000/06/21 09:41
4	L4	2	2 and 3	USPAT	2000/06/21 09:41

USPT

US-CL-CURRENT: 435/108,435/200 ,536/23.7 ,536/24.1

US-PAT-NO: 5985617
DOCUMENT-IDENTIFIER: US 5985617 A
TITLE: Microorganisms and methods for overproduction of DAHP by cloned PPS gene
DATE-ISSUED: November 16, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Liao; James C.	Los Angeles	CA	90024	N/A

US-CL-CURRENT: 435/72,435/108 ,435/200 ,536/23.7 ,536/24.1
ABSTRACT:

Genetic elements comprising expression vectors and a gene coding for phosphoenol pyruvate synthase is utilized to enhance diversion of carbon resources into the common aromatic pathway and pathways branching therefrom. The overexpression of phosphoenol pyruvate synthase increases DAHP production to near theoretical yields.

29 Claims, 14 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 6

BSPR:
The overproduction of transketolase in tkt transformed cells has been found to provide an **increased flow of carbon** resources into the common aromatic pathway relative to carbon resource utilization in whole cells that do not harbor such genetic elements. However, the **increased carbon flux** may be further enhanced by additional manipulation of the host strain.

BSPR:
The present invention further provides a method for **increasing carbon flow** for the biosynthesis of DAHP in a host cell comprising the steps of transforming into the host cell recombinant DNA comprising a pps gene so that Pps is expressed at enhanced levels relative to wild type host cells, concentrating the transformed cells through centrifugation, resuspending the cells in a minimal, nutrient lean medium, fermenting the resuspended cells, and isolating DAHP from the medium.

BSPR:
The present invention further provides methods of **increasing carbon flow** into the common aromatic pathway of a host cell comprising the step of transforming the host cell with recombinant DNA comprising a pps gene so that Pps is expressed at appropriate point in the metabolic pathways at enhanced levels relative to wild type host cells.

DEPR:
The inventor have found that cell lines can be developed that **increase the carbon flux** into DAHP production and achieve near theoretical yields of DAHP by overexpressing phosphoenolpyruvate synthase (Pps) in the cell lines. Overexpression of Pps can increase the final concentration and yield of DAHP by as much as two-fold, to a near theoretical maximum as compared to wild type cell lines. The overexpression of Pps is achieved by transforming a cell line with recombinant DNA comprising a pps gene so that Pps is expressed at enhanced level relative to the wild type cell line and so that the yield of DAHP approaches its theoretical value.

DEPR:
Besides the use of the pps gene, the present invention also provides for transfer of genetic elements comprising the tkt gene, the gene coding for DAHP synthase (aroF in E. coli), the gene coding for 3-dehydroquinate synthase (aroB in E. coli), or other genes encoding enzymes that catalyze reactions in the common aromatic pathway. Such a cell transformation can be achieved by transferring one or more plasmids that contain genes that code for enzymes that **increase the carbon flux** for DAHP synthesis and for subsequent synthesis of

other desired cyclic, pre-aromatic, and aromatic metabolites. As a result of this transfer of genetic element(s), more carbon enters and moves through the common aromatic pathway relative to wild type host cells not containing the genetic elements of the present invention.

DEPR:
In one embodiment, the present invention comprises a method for **increasing carbon flow** into the common aromatic pathway of a host cell by increasing the production of DAHP through the overexpression of Pps at the appropriate point in the common aromatic pathway to provide additional PEP at the point where PEP condenses with E4P. **Increasing carbon flow** requires the step of transforming the host cell with recombinant DNA containing a pps genes so that Pps is overexpressed at enhanced levels relative to wild type host cells. DAHP is then produced by fermentation of the transformed cell in a nutrient medium which the DAHP can be extracted from the medium on a batch wise or continuous extraction procedure.

DEPR:
Another embodiment of the present invention is a method for enhancing a host cell's biosynthetic production of compounds derived from the common aromatic pathway. This method involves the step of increasing expression of Pps in the host cell relative to a wild type host cell. The step of increasing expression of Pps can include transferring into the host cell a vector carrying the pps gene. The overexpression of Pps results in forcing **increased carbon flow** into the biosynthesis of DAHP.

DEPR:
Besides being used in the biosynthesis of DAHP, PEP is used as a phosphate donor in the phosphotransferase system (PTS) which is responsible for glucose uptake. Additionally, PEP can be converted to pyruvate by pyruvate kinases and to oxaloacetate by phosphoenolpyruvate carboxylase. All such competing pathways limit the **availability of PEP** for the biosynthesis of DAHP and all metabolites derived from the common aromatic pathway or pathways branching therefrom.

DEPR:
A key component of the methods of the present invention directed at **increased carbon flux** commitment to DAHP and DAHP metabolites is the recycling of pyruvate to PEP. Pyruvate is available in host cells as an end product of glycolysis.

DEPR:
This example demonstrates that the E. coli AB2847 is unable to utilize DAHP, and accumulates DAHP in the medium if DAHP synthase is overexpressed. This strain was used as a host for detecting the flux committed to the aromatic pathways. Since Draths et al. (Draths, K. M., D. L. Pompliano, D. L. Conley, J. W. Frost, A. Berry, G. L. Disbrow, R. J. Staversky, and J. C. Lievense, "Biocatalytic synthesis of aromatics from D-glucose: The role of transketolase," J. Am. Chem. Soc., 1992, 114, 3956-3962) have shown a possible limitation in the production of DAHP by E4P, pAT1 (containing both aroG.sup.fbr and tktA) was transformed into AB2847 to eliminate the limitation of E4P. To test whether **PEP supply limits DAHP production, PEP** synthase (Pps) was overexpressed in AB2847/pAT1 by transforming plasmid pPS341 into this strain. 20-70 copies of the pps gene were expressed in the host cells. As a control, pPS341 was substituted by pPS341X1, which encodes an inactive, but stable pps gene product. The use of the inactive Pps control allowed discrimination between the effect of Pps activity and that of protein overexpression. AB2847/pAT1/pUHE23-2 and AB2847/pAT1 were also used without any other plasmid as additional controls to identify the effect of the cloning vector, pUHE23-2, on DAHP production.

DEPR:
To determine whether the Pps effect requires overexpressed transketolase (Tkt) as well, plasmid pRW5, which contains only aroG.sup.fbr, was used in place of pAT1 in the above experiments. It was found that overproduction of Pps did not increase the DAHP production (FIG. 2A) without the elevated Tkt activity. Therefore, as limitation of small molecules in the biosynthesis of DAHP is concerned, the first limitation arises from the supply of E4P. This bottleneck

shifts to the **supply of PEP** when Tkt is overexpressed, which is believed to increase the supply of E4P.

DEPR:
Since PEP is also converted to OAA by Ppc, the deletion of this enzyme may increase the **supply of PEP**. Therefore, the ppc gene on the chromosome of AB2847 was inactivated to determine whether DAHP production could be increased without Pps overexpression. This was done by transducing AB2847 with a P1 lysate grown on JCL1242 ppc::Km. The resulting transductant, JCL1283 aroB ppc::Km was then transformed with pAT1 or pRW5 and tested for DAHP production in the re-suspension culture as described above. To avoid limitation of OAA in the ppc strain, the culture medium was supplemented with succinate, which was shown to have no effect on DAHP production (data not shown). Contrary to the expectation, ppc mutation did not increase the production of DAHP (FIG. 2B), suggesting that the metabolic flux from PEP to OAA was not significant under the experimental conditions tested here. In fact, the ppc mutation actually decreased the DAHP production for unknown reasons.

USPT

US-CL-CURRENT: 435/108,435/200 ,536/23.7 ,536/24.1

US-PAT-NO: 5906925
DOCUMENT-IDENTIFIER: US 5906925 A
TITLE: Microorganisms and methods for overproduction of DAHP by cloned pps gene
DATE-ISSUED: May 25, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Liao; James C.	N/A	N/A	N/A	N/A
	College Station	TX	77843-312 2	

US-CL-CURRENT: 435/72,435/108 ,435/200 ,536/23.7 ,536/24.1
ABSTRACT:
Genetic elements comprising expression vectors and a gene coding for phosphoenol pyruvate synthase is utilized to enhance diversion of carbon resources into the common aromatic pathway and pathways branching therefrom. The overexpression of phosphoenol pyruvate synthase increases DAHP production to near theoretical yields.
28 Claims, 14 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 6

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BSPR:
The present invention further provides a method for **increasing carbon flow** for the biosynthesis of DAHP in a host cell comprising the steps of transforming into the host cell recombinant DNA comprising a pps gene so that Pps is expressed at enhanced levels relative to wild type host cells, concentrating the transformed cells through centrifugation, resuspending the cells in a minimal, nutrient lean medium, fermenting the resuspended cells, and isolating DAHP from the medium.

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Besides the use of the pps gene, the present invention also provides for transfer of genetic elements comprising the tkt gene, the gene coding for DAHP synthase (aroF in E. coli), the gene coding for 3-dehydroquinate synthase (aroB in E. coli), or other genes encoding enzymes that catalyze reactions in the common aromatic pathway. Such a cell transformation can be achieved by

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In one embodiment, the present invention comprises a method for **increasing carbon flow** into the common aromatic pathway of a host cell by increasing the production of DAHP through the overexpression of Pps at the appropriate point in the common aromatic pathway to provide additional PEP at the point where PEP condenses with E4P. **Increasing carbon flow** requires the step of transforming the host cell with recombinant DNA containing a pps gene so that Pps is overexpressed at enhanced levels relative to wild type host cells. DAHP is then produced by fermentation of the transformed cell in a nutrient medium from which the DAHP can be extracted from the medium on a batch wise or continuous extraction procedure.

DEPR:
Another embodiment of the present invention is a method for enhancing a host cell's biosynthetic production of compounds derived from the common aromatic pathway. This method involves the step of increasing expression of Pps in the host cell relative to a wild type host cell. The step of increasing expression of Pps can include transferring into the host cell a vector carrying the pps gene. The overexpression of Pps results in forcing **increased carbon flow** into the biosynthesis of DAHP.

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A key component of the methods of the present invention directed at **increased carbon flux** commitment to DAHP and DAHP metabolites is the recycling of pyruvate to PEP. Pyruvate is available in host cells as an end product of glycolysis.

DEPR:
This example demonstrates that the E. coli AB2847 is unable to utilize DAHP, and accumulates DAHP in the medium if DAHP synthase is overexpressed. This strain was used as a host for detecting the flux committed to the aromatic pathways. Since Draths et al. (Draths, K. M., D. L. Pompliano, D. L. Conley, J. W. Frost, A. Berry, G. L. Disbrow, R. J. Staversky, and J. C. Lievense, "Biocatalytic synthesis of aromatics from D-glucose: The role of transketolase," J. Am. Chem. Soc., 1992, 114, 3956-3962) have shown a possible limitation in the production of DAHP by E4P, pAT1 (containing both aroG.sup.fbr and tktA) was transformed into AB2847 to eliminate the limitation of E4P. To test whether **PEP supply limits DAHP production, PEP** synthase (Pps) was overexpressed in AB2847/pAT1 by transforming plasmid pPS341 into this strain. 20-70 copies of the pps gene were expressed in the host cells. As a control, pPS341 was substituted by pPS341X1, which encodes an inactive, but stable pps gene product. The use of the inactive Pps control allowed discrimination between the effect of Pps activity and that of protein overexpression. AB2847/pAT1/pUHE23-2 and AB2847/pAT1 were also used without any other plasmid as additional controls to identify the effect of the cloning vector, pUHE23-2, on DAHP production.

DEPR:
To determine whether the Pps effect requires overexpressed transketolase (Tkt) as well, plasmid pRW5, which contains only aroG.sup.fbr, was used in place of pAT1 in the above experiments. It was found that overproduction of Pps did not increase the DAHP production (FIG. 2A) without the elevated Tkt activity.

Therefore, as limitation of small molecules in the biosynthesis of DAHP is concerned, the first limitation arises from the supply of E4P. This bottleneck shifts to the **supply of PEP** when Tkt is overexpressed, which is believed to increase the supply of E4P.

DEPR:
Since PEP is also converted to OAA by Ppc, the deletion of this enzyme may increase the **supply of PEP**. Therefore, the ppc gene on the chromosome of AB2847 was inactivated to determine whether DAHP production could be increased without Pps overexpression. This was done by transducing AB2847 with a P1 lysate grown on JCL1242 ppc::Km. The resulting transductant, JCL1283 aroB ppc::Km was then transformed with pAT1 or pRW5 and tested for DAHP production in the re-suspension culture as described above. To avoid limitation of OAA in the ppc strain, the culture medium was supplemented with succinate, which was shown to have no effect on DAHP production (data not shown). Contrary to the expectation, ppc mutation did not increase the production of DAHP (FIG. 2B), suggesting that the metabolic flux from PEP to OAA was not significant under the experimental conditions tested here. In fact, the ppc mutation actually decreased the DAHP production for unknown reasons.

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	2792	carbon near2 (flux or flow)	USPAT	2000/06/21 09:22
2	L2	133	1 near4 (modif$8 or alter$8 or increas$8)	USPAT	2000/06/21 09:25
3	L3	100	(phosphoenol adj pyruvate or pep or phosphoenolpyruvate or phospho adj enol adj pyruvate) near4 (suppl$4 or availab$8)	USPAT	2000/06/21 09:41
4	L4	2	2 and 3	USPAT	2000/06/21 09:51
5	L5	2011	phosphotransferase$1 or phospho adj transferase$1	USPAT	2000/06/21 09:53
6	L6	6	(2 or 3) and 5	USPAT	2000/06/21 09:58
7	L7	16	(2 or 3) same (aromatic or shikimate)	USPAT	2000/06/21 09:59

USPT

US-CL-CURRENT: 435/108,435/200 ,536/23.7 ,536/24.1

US-PAT-NO: 5985617
DOCUMENT-IDENTIFIER: US 5985617 A
TITLE: Microorganisms and methods for overproduction of DAHP by cloned PPS gene
DATE-ISSUED: November 16, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Liao; James C.	Los Angeles	CA	90024	N/A

US-CL-CURRENT: 435/72,435/108 ,435/200 ,536/23.7 ,536/24.1
ABSTRACT:

Genetic elements comprising expression vectors and a gene coding for phosphoenol pyruvate synthase is utilized to enhance diversion of carbon resources into the common aromatic pathway and pathways branching therefrom. The overexpression of phosphoenol pyruvate synthase increases DAHP production to near theoretical yields.

29 Claims, 14 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 6

BSPR:
The overproduction of transketolase in tkt transformed cells has been found to provide an **increased flow of carbon** resources into the common aromatic pathway relative to carbon resource utilization in whole cells that do not harbor such genetic elements. However, the **increased carbon flux** may be further enhanced by additional manipulation of the host strain.

BSPR:
The present invention further provides a method for **increasing carbon flow** for the biosynthesis of DAHP in a host cell comprising the steps of transforming into the host cell recombinant DNA comprising a pps gene so that Pps is expressed at enhanced levels relative to wild type host cells, concentrating the transformed cells through centrifugation, resuspending the cells in a minimal, nutrient lean medium, fermenting the resuspended cells, and isolating DAHP from the medium.

BSPR:
The present invention further provides methods of **increasing carbon flow** into the common aromatic pathway of a host cell comprising the step of transforming the host cell with recombinant DNA comprising a pps gene so that Pps is expressed at appropriate point in the metabolic pathways at enhanced levels relative to wild type host cells.

DEPR:
The inventor have found that cell lines can be developed that **increase the carbon flux** into DAHP production and achieve near theoretical yields of DAHP by overexpressing phosphoenolpyruvate synthase (Pps) in the cell lines. Overexpression of Pps can increase the final concentration and yield of DAHP by as much as two-fold, to a near theoretical maximum as compared to wild type cell lines. The overexpression of Pps is achieved by transforming a cell line with recombinant DNA comprising a pps gene so that Pps is expressed at enhanced level relative to the wild type cell line and so that the yield of DAHP approaches its theoretical value.

DEPR:
Besides the use of the pps gene, the present invention also provides for transfer of genetic elements comprising the tkt gene, the gene coding for DAHP synthase (aroF in E. coli), the gene coding for 3-dehydroquinate synthase (aroB in E. coli), or other genes encoding enzymes that catalyze reactions in the common aromatic pathway. Such a cell transformation can be achieved by transferring one or more plasmids that contain genes that code for enzymes that **increase the carbon flux** for DAHP synthesis and for subsequent synthesis of

other desired cyclic, pre-aromatic, and aromatic metabolites. As a result of this transfer of genetic element(s), more carbon enters and moves through the common aromatic pathway relative to wild type host cells not containing the genetic elements of the present invention.

DEPR:
In one embodiment, the present invention comprises a method for **increasing carbon flow** into the common aromatic pathway of a host cell by increasing the production of DAHP through the overexpression of Pps at the appropriate point in the common aromatic pathway to provide additional PEP at the point where PEP condenses with E4P. **Increasing carbon flow** requires the step of transforming the host cell with recombinant DNA containing a pps genes so that Pps is overexpressed at enhanced levels relative to wild type host cells. DAHP is then produced by fermentation of the transformed cell in a nutrient medium which the DAHP can be extracted from the medium on a batch wise or continuous extraction procedure.

DEPR:
Another embodiment of the present invention is a method for enhancing a host cell's biosynthetic production of compounds derived from the common aromatic pathway. This method involves the step of increasing expression of Pps in the host cell relative to a wild type host cell. The step of increasing expression of Pps can include transferring into the host cell a vector carrying the pps gene. The overexpression of Pps results in forcing **increased carbon flow** into the biosynthesis of DAHP.

DEPR:
Besides being used in the biosynthesis of DAHP, PEP is used as a phosphate donor in the **phosphotransferase** system (PTS) which is responsible for glucose uptake. Additionally, PEP can be converted to pyruvate by pyruvate kinases and to oxaloacetate by phosphoenolpyruvate carboxylase. All such competing pathways limit the **availability of PEP** for the biosynthesis of DAHP and all metabolites derived from the common aromatic pathway or pathways branching therefrom.

DEPR:
A key component of the methods of the present invention directed at **increased carbon flux** commitment to DAHP and DAHP metabolites is the recycling of pyruvate to PEP. Pyruvate is available in host cells as an end product of glycolysis.

DEPR:
As shown in FIG. 3A, for maximum yield of DAHP production by strains without Pps overproduction, 7 moles of glucose are needed to produce 3 moles of DAHP (43% molar yield) and 7 moles of pyruvate which is further metabolized. The relative flux through each intermediate step is also shown in FIG. 3A. The formation of pyruvate is necessary because of the stoichiometry of the **phosphotransferase** system for glucose uptake.

DEPR:
The stimulation of glucose consumption in the previous work was attributed to the altered PEP/pyruvate ratio. It was hypothesized that increased PEP/pyruvate ratio stimulates the **phosphotransferase** system for increased glucose consumption, which in turn results in the excretion of pyruvate.

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This example demonstrates that the E. coli AB2847 is unable to utilize DAHP, and accumulates DAHP in the medium if DAHP synthase is overexpressed. This strain was used as a host for detecting the flux committed to the aromatic pathways. Since Draths et al. (Draths, K. M., D. L. Pompliano, D. L. Conley, J. W. Frost, A. Berry, G. L. Disbrow, R. J. Staversky, and J. C. Lievense, "Biocatalytic synthesis of aromatics from D-glucose: The role of transketolase," J. Am. Chem. Soc., 1992, 114, 3956-3962) have shown a possible limitation in the production of DAHP by E4P, pAT1 (containing both aroG.sup.fbr and tktA) was transformed into AB2847 to eliminate the limitation of E4P. To test whether **PEP supply limits DAHP production, PEP** synthase (Pps) was overexpressed in AB2847/pAT1 by transforming plasmid pPS341 into this strain. 20-70 copies of the pps gene were expressed in the host cells. As a control,

pPS341 was substituted by pPS341X1, which encodes an inactive, but stable pps gene product. The use of the inactive Pps control allowed discrimination between the effect of Pps activity and that of protein overexpression. AB2847/pAT1/pUHE23-2 and AB2847/pAT1 were also used without any other plasmid as additional controls to identify the effect of the cloning vector, pUHE23-2, on DAHP production.

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USPT

US-CL-CURRENT: 435/108,435/200 ,536/23.7 ,536/24.1

US-PAT-NO: 5906925
DOCUMENT-IDENTIFIER: US 5906925 A
TITLE: Microorganisms and methods for overproduction of DAHP by cloned pps gene
DATE-ISSUED: May 25, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Liao; James C.	N/A	N/A	N/A	N/A
	College Station	TX	77843-312 2	

US-CL-CURRENT: 435/72,435/108 ,435/200 ,536/23.7 ,536/24.1
ABSTRACT:
Genetic elements comprising expression vectors and a gene coding for phosphoenol pyruvate synthase is utilized to enhance diversion of carbon resources into the common aromatic pathway and pathways branching therefrom. The overexpression of phosphoenol pyruvate synthase increases DAHP production to near theoretical yields.
28 Claims, 14 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 6

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BSPR:
The present invention further provides a method for **increasing carbon flow** for the biosynthesis of DAHP in a host cell comprising the steps of transforming into the host cell recombinant DNA comprising a pps gene so that Pps is expressed at enhanced levels relative to wild type host cells, concentrating the transformed cells through centrifugation, resuspending the cells in a minimal, nutrient lean medium, fermenting the resuspended cells, and isolating DAHP from the medium.

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overexpressed in AB2847/pAT1 by transforming plasmid pPS341 into this strain. 20-70 copies of the pps gene were expressed in the host cells. As a control, pPS341 was substituted by pPS341X1, which encodes an inactive, but stable pps gene product. The use of the inactive Pps control allowed discrimination between the effect of Pps activity and that of protein overexpression. AB2847/pAT1/pUHE23-2 and AB2847/pAT1 were also used without any other plasmid as additional controls to identify the effect of the cloning vector, pUHE23-2, on DAHP production.

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USPT

US-CL-CURRENT: 530/370,536/23.6 ,800/278 ,800/290 ,800/298

US-PAT-NO: 5891726
DOCUMENT-IDENTIFIER: US 5891726 A
TITLE: Procedure to increase the seed productivity of plants and to accelerate the growth of plants by means of an additional plastidic pyruvate, phosphate dikinase
DATE-ISSUED: April 6, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sheriff; Ahmed	D-12305 Berlin	N/A	N/A	DEX

US-CL-CURRENT: 435/419,530/370 ,536/23.6 ,800/278 ,800/290 ,800/298
ABSTRACT:
The present invention includes novel methods for increasing the seed productivity or accelerating the growth rate of a plant and plants produced by such methods. Such plants have at least one cell transformed with an expression complex comprising a promoter operably linked to a gene encoding a pyruvate phosphate dikinase which is capable of converting pyruvate into phosphoenolpyruvate. The plants are made by transforming at least one plant cell with an appropriate expression construct, regenerating plants from one or more transformed plant cells and selecting at least one plant having the desired phenotype.
7 Claims, 16 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 8

BSPR:
Latzko and Kelly (1983) suggested another ten biochemical reason for the occurrence of PEPCase. But, these other purposes do not bring about immediate energetical advantages. Phosphoenolpyruvate is a substrate for PEPCase. It can, for example, be generated from pyruvate by PPDK and, maybe PEPCase is limited by the **supply of phosphoenolpyruvate**. The enzymatic characteristics and metabolic functions of PPDK from non-photosynthetically active tissues are only poorly understood (Fi.beta.lthaler et al. 1995; Rosche et al. 1994).

DRPR:
FIG. 3: Schematic drawing of the binary vectors with the M. crystallinum PPDK-insertion. .DELTA. represents a deletion of 183 bp at the 5'-end. The PPDK cDNA-sequence from M. crystallinum is 3173 bp long (Fi.beta.lthaler et al. 1995). The transnational start-codon is at position 63. The termination codon is at position 1920. The protein deduced from the cDNA sequence contains 949 amino acids (aa), which corresponds to a relative molecular mass (M.sub.r) of 103244 daltons. The presequence has a length of 74 aa. The mature plastidic enzyme has a M.sub.r of 94 kDa. 5'- and 3'-untranslated parts of the cDNA are indicated as terminal hatched boxes (left 5'-, right 3'-untranslated region; the dotted box represents the plastid presequence with three ATGs in the open reading frame). The T-DNA contained a neomycin **phosphotransferase** (nptII, kanamycin resistance) with plant regulatory elements. 35S CaMV: promoter of the cauliflower mosaic virus; ocs polyA: polyadenylation signal of the octopin synthase gene from A. tumefaciens; BR, BL: right, left borders of the T-DNA of Agrobacterium tumefaciens; Km: kanamycin resistance with prokaryotic regulational elements (Beven 1984). Transformation of the tobacco plants by A. tumefaciens was performed according to Horsch et al. (1985).

USPT

US-CL-CURRENT: 435/419,435/468 ,435/469 ,435/69.1 ,536/23.2 ,536/23.6 ,536/24.1

US-PAT-NO: 5856177
DOCUMENT-IDENTIFIER: US 5856177 A
TITLE: Promoters derived from the maize phosphoenolpyruvate carboxylase gene involved in C.sub.4 photosynthesis
DATE-ISSUED: January 5, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Grula; John W.	Pasadena	CA	N/A	N/A
Hudspeth; Richard L.	Altadena	CA	N/A	N/A

US-CL-CURRENT: 435/320.1,435/419 ,435/468 ,435/469 ,435/69.1 ,536/23.2 ,536/23.6 ,536/24.1

ABSTRACT:
A plasmid comprising a phosphoenolpyruvate carboxylase gene and promoter isolated from maize. The phosphoenolpyruvate carboxylase gene encodes a phosphoenolpyruvate carboxylase isozyme involved in C.sub.4 photosynthesis and which is not expressed in seeds.
6 Claims, 36 Drawing figures
Exemplary Claim Number: 2
Number of Drawing Sheets: 30

DEPR:
Alternatively, the suspension may be resuspended in fresh medium containing cefotaxime and allowed to grow an additional 4 to 28 days prior plating on solidified medium in Petri dishes. Cell concentration is 1 vol. of suspension cells plus 3 vol. of medium with cefotaxime. Kanamycin at 10 to 300 mg/l preferably about 20 to 200 mg/l more preferably about 40 to 80 mg/l is included in the medium for selection of transformed cells expressing the neomycin **phosphotransferase** (NPT) gene. Cells and embryos proliferating in the selective concentration of kanamycin are further grown as set forth above to mature somatic embryos capable of germinating and regenerating into whole plants according to the procedures described herein.

DEPR:
Immunoprecipitation of in vitro translation products with Staphylococcus aureus Cowan 1 strain cells (IgGsorb supplied by The Enzyme Center, Boston, Mass.) was performed as described by Cullen et al. (1976) J. Immunol. 117 136-142, which is incorporated herein by reference. Antibodies against maize **PEP carboxylase (supplied** by Sigma Chemical Corp. St Louis, Mo.) were prepared by Antibodies Inc. (Davis, Calif.). Proteins were analyzed by SDS polyacrylamide gel electrophoresis in 5-15% gradient slab gels run at 3 V/cm for 16 hr. The gels were treated with EN.sup.3 HANCE (supplied by New England Nuclear), dried and exposed for 16-72 hr to X-ray film at -70.degree. C. with an intensifying screen.

DEPR:
Two T-DNA PstI cleaved right border sequences from A. tumefaciens (strain C-58) were further subdivided with BamHI for integration in the plant genome, a passenger maize phosphoenolpyruvate carboxylase gene described above as the insert of H1.lambda.14, and a chimeric gene (NOS/NPT/TK) capable of expression in plants and conferring resistance to the antibiotics kanamycin and G418 were ligated into pRK290 which contains a wide host range replicon required for replication in A. tumefaciens. This chimeric gene utilizes a nopaline synthetase promoter, the neomycin **phosphotransferase** II coding region from Tn5, and the terminator from the herpes simplex virus thymidine kinase gene. The resultant plasmid, designated DEI PEP 10, is shown in FIG. 33. The complete DEI PEP 10 is given in Hudspeth (1988, Ph.D. Thesis) and incorporated herein by reference.

DEPR:
The suspension culture as obtained in Example 12 was transformed using an Agrobacteria which contained the T-DNA containing binary vector pCIB10

[Rothstein et al., Gene 53 153-161 (1987), incorporated herein by reference] as well as the pAL4404 vir-plasmid. The T-DNA of pCIB10 contains a chimeric gene composed of the promoter from nopaline synthase, the coding region from Tn5 encoding the enzyme neomycin phosphotransferase, and the terminator from nopaline synthase. The Agrobacteria containing pCIB10 were grown on YEB medium containing kanamycin (50 .mu.g/ml). Transformation was accomplished in the same manner as in Example 13 except that the 1 ml aliquots resulting in cells and Agrobacteria were immediately plated on selective media containing either kanamycin (50 .mu.g/ml) or G418 (25 .mu.g/ml). Expression of the nos/neo/nos chimeric gene in transformed plant tissue allows the selection of this tissue in the presence of both antibiotics. The existence in two to four weeks of transformed tissue became apparent on the selection plates. Uninfected tissue as well as added control tissue showed no signs of growth, turned brown and died. Transformed tissue grew very well in the presence of both kanamycin and G418.

DEPR:
The procedure of Example 13 was followed, except that the transforming Agrobacteria used contained the T-DNA vector DEI PEP10 as well as the pAL4404 vir plasmid. DEI PEP10, shown in FIG. 33, utilizes two T-DNA PstI cleaved right border sequences from A. tumefaciens (strain C-58) which had been further subdivided with BamHI for integration in the plant genome, a passenger maize phosphoenolpyruvate carboxylase gene (Pepcase gene), and a chimeric gene (NOS/NPT/TK) capable of expression in plants and conferring resistance to the antibiotics kanamycin and G418. This chimeric gene utilizes a nopaline synthetase promoter, the neomycin phosphotransferase II coding region from Tn5, and the terminator from the herpes simplex virus thymidine kinase gene. Following transformation, embryogenic callus and embryos were obtained by selection on kanamycin (50 mg/l). No resistant callus was obtained from the control (non-transformed callus) plated on kanamycin at this level (50 mg/l).

DEPR:
The procedure of Example 13 was followed, except that the transforming Agrobacteria used contained the T-DNA vector pPMG85/587 [Fillatti et al., Mol. Gen. Genet. 206 192-199 (1987) incorporated herein by reference] as well as the pAL4404 vir plasmid. The plasmid pPMG85/587 carries three chimeric genes capable of expression in plants. Two genes code for neomycin phosphotransferase (NPT) which confers resistance to the antibiotics kanamycin and G418. The third chimeric gene, containing the coding sequence from a mutant aroA gene of S. typhimurium, confers tolerance to the herbicide glyphosate [Comai et al., Science 221 370-371 (1983), incorporated herein by reference]. The Agrobacteria containing pPMG85/587 were grown on medium containing kanamycin (100 .mu.g/ml). Transformation is accomplished as detailed in Example 13 except that the suspension is allowed to grow for 28 days at which time 1 ml aliquots were plated on medium containing selective antibiotics. Expression of the NPT chimeric gene in transformed plant tissue allowed selection of this tissue on both antibiotics. In this instance the selective antibiotic was kanamycin (50 .mu.g/ml).

DEPR:
The transformation procedure of Example 13 was followed except there was used as the transforming Agrobacteria one containing the T-DNA binary vector pCIB715 [Rothstein et al. Gene 53 153-161 (1987)] as well as the vir plasmid. The T-DNA of pCIB715 contains a chimeric gene composed of the promoter and terminator from the cauliflower mosaic virus (CaMV) 35S transcript [Odell et al., Nature 313 810-812 (1985), incorporated herein by reference] and the coding sequence for hygromycin B phosphotransferase [Gritz et al., Gene 25 179-188 (1983) incorporated herein by reference]. Agrobacteria containing pCIB715 was grown on YEB containing kanamycin (50 .mu.g/ml).

DEPR:
A plasmid containing the gene for expression of kanamycin resistance in plants was constructed (see FIGS. 22 and 23). Plasmid Bin6 obtained from Dr. M. Bevan, Plant Breeding Institute, Cambridge, UK. This plasmid is described in the reference by Bevan [Nucl. Acids Res. 12 8711-8721 (1984) incorporate herein by reference]. Plasmid Bin6 DNA was digested with EcoRI and HindIII and the fragment approximately 1.5 kb in size containing the chimeric neomycin phosphotransferase (NPT) gene is isolated and purified following agarose gel electrophoresis. This fragment was then mixed with plasmid pUC18 DNA which had

been cleaved with endonucleases EcoRI and HindIII. Following incubation with T4 DNA ligase, the resulting DNA was transformed into E. coli strain HB101. The resulting plasmid is called pUC18/neo. This plasmid DNA containing an unwanted BamHI recognition sequence between the neomycin **phosphotransferase** gene and the terminator sequence for nopaline synthase [see Bevan Nucl. Acids Res. 12 8711-8721 (1984) incorporated herein by reference]. To remove this recognition sequence, plasmid pUC18/neo was digested with endoruclease BamHI, followed by treatment with the large fragment of DNA polymerase to create flush ends. The fragment was then incubated with T4 DNA ligase to recircularize the fragment, and transformed into E. coli strain HB101. The resulting plasmid, pUC18/neo(Bam) has lost the BamHI recognition sequence.

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	2792	carbon near2 (flux or flow)	USPAT	2000/06/21 09:22
2	L2	133	1 near4 (modif$8 or alter$8 or increas$8)	USPAT	2000/06/21 09:25
3	L3	100	(phosphoenol adj pyruvate or pep or phosphoenolpyruvate or phospho adj enol adj pyruvate) near4 (suppl$4 or availab$8)	USPAT	2000/06/21 09:41
4	L4	2	2 and 3	USPAT	2000/06/21 09:51
5	L5	2011	phosphotransferase$1 or phospho adj transferase$1	USPAT	2000/06/21 09:53
6	L6	6	(2 or 3) and 5	USPAT	2000/06/21 09:58
7	L7	16	(2 or 3) same (aromatic or shikimate)	USPAT	2000/06/21 09:59

USPT

US-CL-CURRENT: 435/136,435/146 ,435/155 ,435/156 ,435/252.8 ,435/317.1 ,435/320.1 ,435/849

US-PAT-NO: 6030819
DOCUMENT-IDENTIFIER: US 6030819 A
TITLE: Genetically engineered microorganisms and method for producing 4-hydroxybenzoic acid
DATE-ISSUED: February 29, 2000
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Amaratunga; Mohan	Clifton Park	NY	N/A	N/A
Lobos; John Henry	Ballston Spa	NY	N/A	N/A
Johnson; Bruce Fletcher	Scotia	NY	N/A	N/A
Williams; Eric Douglas	Schenectady	NY	N/A	N/A

US-CL-CURRENT: 435/132,435/136 ,435/146 ,435/155 ,435/156 ,435/252.8 ,435/317.1 ,435/320.1 ,435/849
ABSTRACT:

The present invention pertains to a method for economical biofermentative production of 4-hydroxybenzoic acid (PHB) using genetically engineered E. coli. According to the invention, a plasmid is provided which controls the overexpression of chorismate pyruvate lyase, the bacterial enzyme which catalyzes the production of PHB from chorismate. Mutant E. coli selected with a unique two-step screening assay to overproduce chorismate have been transformed with this plasmid, providing a biocatalyst that efficiently converts glucose to PHB.

17 Claims, 3 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 3

DEPR:
In E. coli, transformation of glucose to PHB involves approximately fifteen different enzymatic steps. FIG. 1 provides a summary of the important intermediates in the pathway. The ubiC gene product, CPL, is a likely candidate for amplification because of the low product turnover of CPL, and because it exists at a major branch in the metabolic pathway. At this point in the biosynthetic pathway, CPL's substrate, chorismate, may be converted to PHB, to prephenic acid (PPA) and the **aromatic** amino acids, or to a variety of other products. Therefore, **increasing the carbon flow of metabolism toward chorismate increases** the substrate available for conversion into PHB, and also increases the substrate available to the **aromatic** amino acid pathway through PPA, and to other products. Thus, cells which produce more chorismate and thus more PHB also produce more **aromatic** amino acids.

USPT

US-CL-CURRENT: 435/108,435/200 ,536/23.7 ,536/24.1

US-PAT-NO: 5985617
DOCUMENT-IDENTIFIER: US 5985617 A
TITLE: Microorganisms and methods for overproduction of DAHP by cloned PPS gene
DATE-ISSUED: November 16, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Liao; James C.	Los Angeles	CA	90024	N/A

US-CL-CURRENT: 435/72,435/108 ,435/200 ,536/23.7 ,536/24.1
ABSTRACT:
Genetic elements comprising expression vectors and a gene coding for phosphoenol pyruvate synthase is utilized to enhance diversion of carbon resources into the common aromatic pathway and pathways branching therefrom. The overexpression of phosphoenol pyruvate synthase increases DAHP production to near theoretical yields.
29 Claims, 14 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 6

BSPR:
The overproduction of transketolase in tkt transformed cells has been found to provide an **increased flow of carbon** resources into the common **aromatic** pathway relative to carbon resource utilization in whole cells that do not harbor such genetic elements. However, the **increased carbon flux** may be further enhanced by additional manipulation of the host strain.

BSPR:
The present invention further provides methods of **increasing carbon flow** into the common **aromatic** pathway of a host cell comprising the step of transforming the host cell with recombinant DNA comprising a pps gene so that Pps is expressed at appropriate point in the metabolic pathways at enhanced levels relative to wild type host cells.

DEPR:
Besides the use of the pps gene, the present invention also provides for transfer of genetic elements comprising the tkt gene, the gene coding for DAHP synthase (aroF in E. coli), the gene coding for 3-dehydroquinate synthase (aroB in E. coli), or other genes encoding enzymes that catalyze reactions in the common **aromatic** pathway. Such a cell transformation can be achieved by transferring one or more plasmids that contain genes that code for enzymes that **increase the carbon flux** for DAHP synthesis and for subsequent synthesis of other desired cyclic, pre-**aromatic, and aromatic** metabolites. As a result of this transfer of genetic element(s), more carbon enters and moves through the common **aromatic** pathway relative to wild type host cells not containing the genetic elements of the present invention.

DEPR:
In one embodiment, the present invention comprises a method for **increasing carbon flow** into the common **aromatic** pathway of a host cell by increasing the production of DAHP through the overexpression of Pps at the appropriate point in the common **aromatic** pathway to provide additional PEP at the point where PEP condenses with E4P. **Increasing carbon flow** requires the step of transforming the host cell with recombinant DNA containing a pps genes so that Pps is overexpressed at enhanced levels relative to wild type host cells. DAHP is then produced by fermentation of the transformed cell in a nutrient medium which the DAHP can be extracted from the medium on a batch wise or continuous extraction procedure.

DEPR:
Another embodiment of the present invention is a method for enhancing a host cell's biosynthetic production of compounds derived from the common **aromatic**

pathway. This method involves the step of increasing expression of Pps in the host cell relative to a wild type host cell. The step of increasing expression of Pps can include transferring into the host cell a vector carrying the pps gene. The overexpression of Pps results in forcing **increased carbon flow** into the biosynthesis of DAHP.

DEPR:
Besides being used in the biosynthesis of DAHP, PEP is used as a phosphate donor in the phosphotransferase system (PTS) which is responsible for glucose uptake. Additionally, PEP can be converted to pyruvate by pyruvate kinases and to oxaloacetate by phosphoenolpyruvate carboxylase. All such competing pathways limit the **availability of PEP** for the biosynthesis of DAHP and all metabolites derived from the common **aromatic** pathway or pathways branching therefrom.

DEPR:
This example demonstrates that the E. coli AB2847 is unable to utilize DAHP, and accumulates DAHP in the medium if DAHP synthase is overexpressed. This strain was used as a host for detecting the flux committed to the **aromatic** pathways. Since Draths et al. (Draths, K. M., D. L. Pompliano, D. L. Conley, J. W. Frost, A. Berry, G. L. Disbrow, R. J. Staversky, and J. C. Lievense, "Biocatalytic synthesis of aromatics from D-glucose: The role of transketolase," J. Am. Chem. Soc., 1992, 114, 3956-3962) have shown a possible limitation in the production of DAHP by E4P, pAT1 (containing both aroG.sup.fbr and tktA) was transformed into AB2847 to eliminate the limitation of E4P. To test whether **PEP supply limits DAHP production, PEP** synthase (Pps) was overexpressed in AB2847/pAT1 by transforming plasmid pPS341 into this strain. 20-70 copies of the pps gene were expressed in the host cells. As a control, pPS341 was substituted by pPS341X1, which encodes an inactive, but stable pps gene product. The use of the inactive Pps control allowed discrimination between the effect of Pps activity and that of protein overexpression. AB2847/pAT1/pUHE23-2 and AB2847/pAT1 were also used without any other plasmid as additional controls to identify the effect of the cloning vector, pUHE23-2, on DAHP production.

USPT

US-CL-CURRENT: 435/108,435/200 ,536/23.7 ,536/24.1

US-PAT-NO: 5906925
DOCUMENT-IDENTIFIER: US 5906925 A
TITLE: Microorganisms and methods for overproduction of DAHP by cloned pps gene
DATE-ISSUED: May 25, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Liao; James C.	N/A	N/A	N/A	N/A
	College Station	TX	77843-312 2	

US-CL-CURRENT: 435/72,435/108 ,435/200 ,536/23.7 ,536/24.1
ABSTRACT:
Genetic elements comprising expression vectors and a gene coding for phosphoenol pyruvate synthase is utilized to enhance diversion of carbon resources into the common aromatic pathway and pathways branching therefrom. The overexpression of phosphoenol pyruvate synthase increases DAHP production to near theoretical yields.
28 Claims, 14 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 6

BSPR:
The overproduction of transketolase in tkt transformed cells has been found to provide an **increased flow of carbon** resources into the common **aromatic** pathway relative to carbon resource utilization in whole cells that do not harbor such genetic elements. However, the **increased carbon flux** may be further enhanced by additional manipulation of the host strain.

BSPR:
The present invention further provides methods of **increasing carbon flow** into the common **aromatic** pathway of a host cell comprising the step of transforming the host cell with recombinant DNA comprising a pps gene so that Pps is expressed at appropriate point in the metabolic pathways at enhanced levels relative to wild type host cells.

DEPR:
Besides the use of the pps gene, the present invention also provides for transfer of genetic elements comprising the tkt gene, the gene coding for DAHP synthase (aroF in E. coli), the gene coding for 3-dehydroquinate synthase (aroB in E. coli), or other genes encoding enzymes that catalyze reactions in the common **aromatic** pathway. Such a cell transformation can be achieved by transferring one or more plasmids that contain genes that code for enzymes that **increase the carbon flux** for DAHP synthesis and for subsequent synthesis of other desired cyclic, pre-**aromatic, and aromatic** metabolites. As a result of this transfer of genetic element(s), more carbon enters and moves through the common **aromatic** pathway relative to wild type host cells not containing the genetic elements of the present invention.

DEPR:
In one embodiment, the present invention comprises a method for **increasing carbon flow** into the common **aromatic** pathway of a host cell by increasing the production of DAHP through the overexpression of Pps at the appropriate point in the common **aromatic** pathway to provide additional PEP at the point where PEP condenses with E4P. **Increasing carbon flow** requires the step of transforming the host cell with recombinant DNA containing a pps gene so that Pps is overexpressed at enhanced levels relative to wild type host cells. DAHP is then produced by fermentation of the transformed cell in a nutrient medium from which the DAHP can be extracted from the medium on a batch wise or continuous extraction procedure.

DEPR:

Another embodiment of the present invention is a method for enhancing a host cell's biosynthetic production of compounds derived from the common **aromatic** pathway. This method involves the step of increasing expression of Pps in the host cell relative to a wild type host cell. The step of increasing expression of Pps can include transferring into the host cell a vector carrying the pps gene. The overexpression of Pps results in forcing **increased carbon flow** into the biosynthesis of DAHP.

DEPR:
Besides being used in the biosynthesis of DAHP, PEP is used as a phosphate donor in the phosphotransferase system (PTS) which is responsible for glucose uptake. Additionally, PEP can be converted to pyruvate by pyruvate kinases and to oxaloacetate by phosphoenolpyruvate carboxylase. All such competing pathways limit the **availability of PEP** for the biosynthesis of DAHP and all metabolites derived from the common **aromatic** pathway or pathways branching therefrom.

DEPR:
This example demonstrates that the E. coli AB2847 is unable to utilize DAHP, and accumulates DAHP in the medium if DAHP synthase is overexpressed. This strain was used as a host for detecting the flux committed to the **aromatic** pathways. Since Draths et al. (Draths, K. M., D. L. Pompliano, D. L. Conley, J. W. Frost, A. Berry, G. L. Disbrow, R. J. Staversky, and J. C. Lievense, "Biocatalytic synthesis of aromatics from D-glucose: The role of transketolase," J. Am. Chem. Soc., 1992, 114, 3956-3962) have shown a possible limitation in the production of DAHP by E4P, pAT1 (containing both aroG.sup.fbr and tktA) was transformed into AB2847 to eliminate the limitation of E4P. To test whether **PEP supply limits DAHP production, PEP** synthase (Pps) was overexpressed in AB2847/pAT1 by transforming plasmid pPS341 into this strain. 20-70 copies of the pps gene were expressed in the host cells. As a control, pPS341 was substituted by pPS341X1, which encodes an inactive, but stable pps gene product. The use of the inactive Pps control allowed discrimination between the effect of Pps activity and that of protein overexpression. AB2847/pAT1/pUHE23-2 and AB2847/pAT1 were also used without any other plasmid as additional controls to identify the effect of the cloning vector, pUHE23-2, on DAHP production.

USPT

US-CL-CURRENT: 536/23.2

US-PAT-NO: 5866396
DOCUMENT-IDENTIFIER: US 5866396 A
TITLE: Microbial production of indigo
DATE-ISSUED: February 2, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Weyler; Walter	San Francisco	CA	94131	N/A
Dodge; Timothy C.	Rochester	NY	14617	N/A
Lauff; John J.	Rochester	NY	14612	N/A
Wendt; Dan J.	San Mateo	CA	94401	N/A

US-CL-CURRENT: 435/195,536/23.2
ABSTRACT:

There is provided an improved process for the biosynthetic production of indigo, the improvement comprising removing unwanted by-products such as isatin or indirubin from the broth in which such indigo is produced. Isatin can be removed by enzymatic activity using an isatin-removing enzyme such as an isatin hydrolase, or by other techniques such as process parameters (elevated temperature, pH), or by contacting the broth containing the isatin with appropriate adsorption compounds/compositions such as carbon or appropriate resins. Since isatin is the precursor of indirubin, the indirubin levels are decreased as a result of isatin removal.

3 Claims, 16 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 13

BSPR:

Tryptophan pathway genes useful in securing biosynthetic indole accumulation include a trp operon, isolated from a microorganism as a purified DNA molecule that encodes an enzymatic pathway capable of directing the biosynthesis of L-tryptophan from chorismic acid. (A. J. Pittard (1987) Biosynthesis of **Aromatic** Amino Acids in Escherichia coli and Salmonella typhimurium, F. C. Neidhardt, ed., American Society for Microbiology, publisher, pp. 368-394.) Indole accumulation is enabled by modification of one or more of the pathway's structural elements and/or regulatory regions. This modified trp operon may then be introduced into a suitable host such as a microorganism, plant tissue culture system or other suitable expression system. It should be noted that the term "indole accumulation" does not necessarily indicate that indole actually accumulates intracellularly. Instead, this term can indicate that there is an **increased flux of carbon** to indole and indole is made available as a substrate for intracellular catalytic reactions such as indoxyl formation and other than the formation of L-tryptophan. In the context of this invention, the "accumulated" indole may be consumed in the conversion of indole to indoxyl by an oxygenase such as the **aromatic** dioxygenase NDO, or an **aromatic** monooxygenase such as TMO, or it may actually build up intracellularly and extracellularly, as would be the case when the desired end product is indole or one of its derivatives.